

Functional Sperm of the Yellowtail (*Seriola quinqueradiata*) Were Produced in the Small-Bodied Surrogate, Jack Mackerel (*Trachurus japonicus*).

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1 **Figure legends**

2 **Fig. 1** Section of the donor testis of the yellowtail stained with hematoxylin and eosin. **a** Overview of
3 the testis. Upper and lower rectangles show the area rich in type A spermatogonia and sperm,
4 respectively. **b** and **c** show higher magnification images of upper and lower rectangles in **a**, respectively.
5 Scale bars: 2 mm (**a**) and 20 μ m (**b** and **c**).

6

7 **Fig. 2** Intraperitoneal transplantation of donor testicular cells into the jack mackerel larvae. **a**
8 Bright-field image. **b** Fluorescent image. **c** Transplanted (TP) and non-transplanted (NTP) individuals.
9 N, transplantation needle. Scale bar = 1 mm.

10

11 **Fig. 3** Incorporation of transplanted PKH26-labeled cells into recipient genital ridges. Fluorescent (**a**,
12 **c**) and yellowtail *vasa in situ* hybridization (**b**, **d**) images of the excised genital ridges from
13 transplanted (**a**, **b**) and nontransplanted (**c**, **d**) fish at 20 dpt. *Arrowheads* indicate donor-derived germ
14 cells that were labeled with PKH-26 (**a**), and were expressing the yellowtail *vasa* mRNA (**b**). Scale bars
15 = 20 μ m.

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17 **Fig. 4** Sections of jack mackerel (**a**, **b**) and the yellowtail (**c**, **d**) testis hybridized with antisense jack
18 mackerel (**a**, **c**) and yellowtail *vasa* probes (**b**, **d**). Scale bars = 20 μ m.

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20 **Fig. 5** RT-PCR amplification of donor-derived yellowtail *vasa* mRNA in recipient gonads.

21 Electrophoresis patterns of RT-PCR of recipient number 1–14 (**a**) and 15–28 (**b**) using specific primers
22 for the yellowtail *vasa* (upper panels) and jack mackerel *vasa* (lower panels) cDNA sequences. *Lane P*
23 is a positive control obtained using cDNA derived from the yellowtail testis as template. *Lane N* is a
24 negative control containing no cDNA template. *M* represents a molecular weight marker.

25

26 **Fig. 6** Detection of the colony containing donor-derived germ cells in recipient testis. Serial sections of
27 testis from jack mackerel testis hybridized with yellowtail (**a**) and jack mackerel (**b**) *vasa* probes and
28 were stained with hematoxylin and eosin (**c**). The colony containing donor-derived germ cells is
29 surrounded with a dotted line. Scale bars = 20 μ m.

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31 **Fig. 7** PCR amplification of donor-derived yellowtail *vasa* genomic DNA fragment in recipient semen.
32 *Lane P* is a positive control obtained using genomic DNA extracted from the yellowtail semen as
33 template. *Lane N* is a negative control containing no DNA template. Electrophoretic mobility of the
34 targeted DNA fragment was indicated by *arrowheads*.

35

36 **Fig. 8** Embryos at 48 h-post-fertilization obtained from progeny tests. **a** Normal embryos obtained by
37 crossing the yellowtail female and male. **b** Eggs obtained by crossing the yellowtail female and male
38 recipient number 28. *Arrowhead* indicates a normal embryo. **c** Eggs obtained by crossing the yellowtail
39 female and wild-type jack mackerel male. Scale bars = 1 mm.

40

41 **Fig. 9** Donor-derived offspring obtained from progeny tests. **a** Newly hatched larva obtained by
42 crossing the yellowtail female and male. **b–d** Newly hatched larvae obtained by crossing the yellowtail
43 female and male recipient number 28. **b** Normal individual. **c, d** Abnormal individuals. Scale bars =
44 500 μm .

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46 **Fig. 10** PCR amplification of *β -actin* and *vasa* genomic DNA fragments in larvae obtained from
47 progeny tests. Electrophoresis patterns of PCR using specific primers for yellowtail *β -actin* (**a**), jack
48 mackerel *β -actin* (**b**), yellowtail *vasa* (**c**), and jack mackerel *vasa* (**d**) DNA sequences. *Lane 1* and *2* are
49 larva samples obtained from the crosses of recipient mackerel number 28 and the yellowtail females.
50 *Lane 3* and *4* are larva samples of wild type yellowtail. *Lane 5* and *6* are wild type jack mackerel. *Lane*
51 *7* is a negative control containing no DNA template. *M* represents a molecular weight marker.

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